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# Obtaining hemicellulosic hydrolysate from corn cobs through acid hydrolysis for xylitol production by *Candida guilliermondii* FTI 20037

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*ABSTRACT*

This study evaluated xylose concentration in relation to hemicellulose consumption during the acid hydrolysis of corn cobs in a 50-liter AISI 316 stainless steel reactor equipped with a jacket for indirect heating by electric resistance. The tests were conducted with a solid/liquid ratio of 1:10, using various concentrations of diluted sulfuric acid (50 to 200 mg of acid per g of dry matter) and temperatures (110 to 150 °C) following a $2^2$ factorial design with 4 central point repetitions and face-centered tests. The acid hydrolysis condition of corn cobs that yielded the highest xylose concentration and hemicellulose consumption (condition of 133 °C and 50 $mg.g^{-1}$) was used for xylitol production by *Candida guilliermondii*. In 72 hours, 94 % of the initial xylose was already consumed, resulting in a medium with almost 40 $g.L^{-1}$ of xylitol, with a yield of 0.66 $g_{xylitol}.g_{xylose}^{-1}$.

**Keywords:** Corn cob. Twin-screw extrusion. Organosolv. Lignin precipitation.

## INTRODUCTION

Brazil has a large variety of agricultural and agro-industrial residues with significant economic and social interest for bioprocessing. These include cereal straw, corn, wheat, corn cobs, sugarcane bagasse, rice husks, and oat husks [1]. Corn cobs, a high-volume solid residue from corn processing, are currently used as animal feed or returned to the soil. Corn cobs, a lignocellulosic material, contain approximately 391 g of cellulose, 421 g of hemicellulose, 91 g of lignin, 17 g of protein, and 12 g of ash per kg of dry matter. They can serve as a renewable biomass source for producing soluble sugars, which can be converted into high-value products like xylitol and ethanol through chemical, enzymatic, and fermentative processes [2].

Acid hydrolysis is the most commonly used method for obtaining hemicellulose hydrolysate from lignocellulosic materials [3]. This process requires high pressure and temperature, generating fermentable sugars, with xylose being the predominant sugar from the hemicellulose fraction [4]. However, it can also release sugar degradation products such as furfural, 5-hydroxymethylfurfural (HMF), and other toxic compounds (formic, acetic, and levulinic acids, and phenolic compounds), which can inhibit the fermentation process [5]. The bioconversion of xylose to xylitol is a crucial pathway within the biorefinery concept.

Xylose-rich lignocellulosic hydrolysates can be used for microbiological xylitol production, offering an alternative to the commercial chemical synthesis process [1]. Several yeasts that use xylose as a carbon source are known ethanol producers; however, under low oxygen conditions, they form byproducts such as xylitol, ribitol, arabitol, glycerol, and acetate. Depending on the cofactor specificity of the first enzyme in xylose metabolism, xylose reductase (EC 1.1.1.21), yeasts like *Candida guilliermondii*, *Debaryomyces hansenii* and *Candida parapsilosis* are considered good xylitol producers. Xylitol is a sweetener with unique properties, such as anti-carcinogenicity and insulin-independent metabolism. Xylitol accumulation is attributed to the redox balance (NADH/NAD+), where higher oxygen availability activates the tricarboxylic acid cycle and NAD+ regeneration [6].

## MATERIAL & METHODS

The corn cob crushed, dried, and bagged (20 kg), sourced from RASUL - Indústria e Comércio de Rações Ltda – PR was used in this project. The hydrolysis of corn cobs was carried out at using an AISI 316 stainless steel reactor with a total volumetric capacity of 50 liters, equipped with a jacket for indirect heating by electric resistance. The acid hydrolyses were conducted according to a $2^2$ factorial design with 4 repetitions at the central point and tests corresponding to the face-centered design. It was used 40 liters of water and 4 kg of dry corn cob for each experiment The independent variables studied in this work were temperature and sulfuric acid and the dependent variable were the hemicellulose conversion into xylose. The temperature varied from 110 to 150 °C, and the sulfuric acid varied from 50 to 200 mg per gram of dry corn cob. The results were analyzed in the Statistica software version 14. All the samples were characterized to obtain the cellulose, hemicellulose and lignin concentrations according to the National Renewable Energy Laboratory.

The optimal condition obtained was concentrated in a vacuum concentrator until 60 $g.L^{-1}$ and was treated by pH adjustment and adsorption with activated charcoal, according to the methodology established by Alves et al. (1997), adjusting the pH to 5.5 with CaO and $H_3PO_4$. The treated hydrolysate was sterilized in an autoclave at 111°C for 15 minutes.

The sterile medium was fermented for xylitol production in a shaker incubator with 200 rpm at 30 °C. The *Candida guilliermondii* FTI 20037 was the yeast used in the fermentation, with initial cell concentration of 0.2 $g.L^{-1}$. The medium was supplemented with 4.5 $g.L^{-1}$ of $(NH_4)_2SO_4$ and 5.0 $g.L^{-1}$ of yeast extract. Samples were extracted every 24 h, until 96 h for sugar, xylitol and cell concentration determination.

# RESULTS & DISCUSSION

Table 1 displays the factorial design, comparing the characterization and the Hemicellulose conversion in xylose of each essay.

| Essay | A* (% w/w) x1 | T* (°C) x2 | Xylose (g.L$^{-1}$) | Glycose (g.L$^{-1}$) | Cellulose (% w/w) | Hemicellulose (% w/w) | Lignin (% w/w) | Ashes (% w/w) | Hemicellulose conversion in xylose (% w/w) |
|---|---|---|---|---|---|---|---|---|---|
| Raw corn cob | | | | | 35,9 ± 3,0 | 36,3 | 16,8 ± 1,0 | 1,7 ± 0,0 | |
| 1 | 50 | 110 | 26.83 | 2.07 | 37.5 ± 2.3 | 8.1 | 12.3 ± 0.7 | 0.3 ± 0.0 | 93.5 |
| 2 | 200 | 110 | 29.61 | 1.91 | 29.4 ± 0.3 | 3.4 | 11.3 ± 0.9 | 0.5 ± 0.0 | 88.4 |
| 3 | 50 | 150 | 23.89 | 2.82 | 41.3 ± 3.2 | 0 | 9.7 ± 0.5 | 0.5 ± 0.0 | 64.5 |
| 4 | 200 | 150 | 8.32 | 6.22 | 26.3 ± 0.2 | 0 | 5.4 ± 0.1 | 0.6 ± 0.0 | 22.4 |
| 5 | 50 | 130 | 33.2 | 2.07 | 33.3 ± 2.4 | 2.4 | 11.0 ± 1.2 | 0.5 ± 0.1 | 96.2 |
| 6 | 200 | 130 | 7.55 | 6.59 | 34.1 ± 2.7 | 0 | 7.4 ± 0.2 | 0.6 ± 0.0 | 20.4 |
| 7 | 125 | 110 | 18.87 | 1.09 | 32.5 ± 0.1 | 4.5 | 8.9 ± 0.3 | 0.6 ± 0.0 | 58.3 |
| 8 | 125 | 150 | 27.35 | 2.14 | 29.4 ± 0.5 | 0 | 6.4 ± 0.4 | 0.5 ± 0.0 | 73.9 |
| CP1* | 125 | 130 | 31.12 | 2.27 | 34.1 ± 1.1 | 0 | 10.4 ± 0.4 | 0.4 ± 0.0 | 84.0 |
| CP2* | 125 | 130 | 29.07 | 1.92 | 41.1 ± 0.4 | 0 | 8.2 ± 0.1 | 0.5 ± 0.0 | 78.5 |
| CP3* | 125 | 130 | 27.15 | 1.83 | 39.5 ± 0.5 | 0 | 9.9 ± 0.0 | 0.5 ± 0.0 | 73.3 |
| CP4* | 125 | 130 | 23.8 | 1.7 | 40.7 ± 0.3 | 0 | 9.3 ± 0.4 | 0.3 ± 0.0 | 64.3 |
| OP** | 50 | 133 | 29 | 1.98 | 38.4 ± 0.8 | 5.2 | 11.6 ± 1.7 | 0.6 ± 0.0 | 91.7 |

**Table 1** Conditions and results of the factorial design with each essays chemical characterization; CP: Center Point; OP: Optimal Condition.

The response factor studied was the hemicellulose conversion, varying between 20.4 and 96.2 % w/w. Meanwhile, the xylose concentration variated between 7.6 and 33.2 g.L$^{-1}$ on the hydrolysate. The two response factors are not linear among each other, once some of the xylose may react to the acid and be converted in degradation sub-products during the acid hydrolysis [7].

The results on the table 1 were analyzed using the software Statistica as a design of experiments by using the model of surface response. As all the experiments were evaluated, it was realized the analysis of variance (ANOVA), generating a model without significant variables. To generate a model with significant variables, the experiments 6 and 8 were removed from the analysis, generating an ANOVA with a correlation coefficient of 90,79%. The confidence level used was 15%, which all the variables were significant. The lack of fit was not significative, once the p-value was higher than 0,15. The equation generated for the model is shown in Equation 1, where the variables are not coded.

$$Hc = -1028 - 0.263\times A + 18.41\times T + 0.00374\times A^2 - 0.072\times T^2 - 0.00617\times A\times T \tag{1}$$

Hc: Hemicellulose conversion (% w/w); A: $H_2SO_4$ concentration (w/w $_{\text{dry mass}}$); T: Temperature (°C);

The range of temperature that obtains the higher values of the response variable is between 118 °C and 133 °C, using only 50 mg.g$^{-1}$ of acid. As the temperature is increased in this range, the concentration of xylose is also increased (experiments 1 and 5 of the table 1), therefore, increasing the productivity. With that in mind, the optimal conditions used in the optimal condition were 50 mg g$^{-1}$ of acid and 133 °C.

The hydrolyzed obtained in the optimal conditions were treated and then fermented using *Candida guilliermondii* FTI 20037 for xylitol production. The results are presented in figure 1.

Although the fermentation time was 96 hours, only with 72 hours of reaction 60.3 g.L$^{-1}$ of the xylose has been consumed, representing 94 % of the initial xylose, reaching 0.55 g.L$^{-1}$.h$^{-1}$ of xylitol productivity in 72 hours. The fermentation process initialized with a cell concentration of 0.2 g.L$^{-1}$, increasing to 6.2 g.L$^{-1}$ at 96 hours of fermentation. The yield of the cell production is 0.09 $g_{cell}.g_{xylose}^{-1}$, and the xylitol production yield was 0.65 $g_{xylitol}.g_{xylose}^{-1}$. Another authors obtained a similar yield of xylitol of 0.66 $g_{xylitol}.g_{xylose}^{-1}$ using hydrolyzed corn cob, however, a productivity of 0.37 g.L$^{-1}$.h$^{-1}$, in a 90 hours fermentation using Candida tropicalis MTCC 6192 [8].

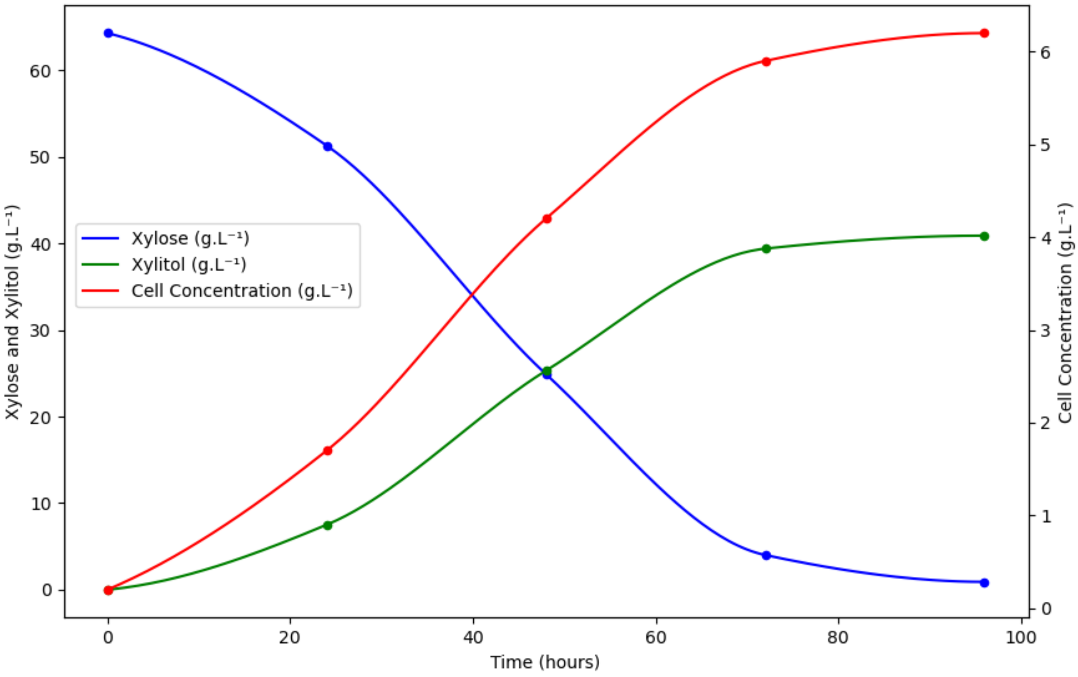

Figure 1: cell growth profile, xylose consumption and xylitol formation

## CONCLUSION

This study effectively identified the optimal conditions for hemicellulose conversion and xylose concentration, providing significant insights into the efficiency and productivity of the acid hydrolysis and fermentation processes. The findings demonstrate a high correlation and yield, aligning well with previous studies, thus contributing valuable knowledge to the field of bioconversion and fermentation technology.

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